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The decidual expression of Interleukin-7 is upregulated in early pregnancy loss

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Abstract

Background: Maternal immunological rejection of the semi-allogenic fetus is discussed as one of the significant factors involved in early pregnancy loss. An array of cytokines secreted by both maternal and fetal cells is involved in generating a delicate maternal immune tolerance. Interleukin-7 (IL-7) is discussed to play a key role in pro-inflammatory processes, but there is still limited insight into the pathophysiological input on placentation and embryonic development in early pregnancy loss.

Patients and methods: Cytokine level differences were identified with quantitative real-time PCR in placental tissue from spontaneous abortions (SA) (n = 18), recurrent spontaneous abortions (RSA) (n = 15), and healthy pregnancies (n = 15) at gestational weeks 7 to 14. Protein expression of IL-7 in the decidua was investigated by immunohistochemistry. IL-7-expressing cells were identified with double-immunofluorescence.

Results: Decidua of women with RSA expressed almost 51-times higher values of IL-7 in gene expression analysis. Immunohistochemistry identified a significant upregulation of IL-7 in the decidua of RSA specimens (p = .013) and in the decidua of women with SA (p = .004). Double-immunofluorescence confirmed decidual stroma cells as IL-7-expressing cells.

Conclusion: Significantly elevated IL-7 values in the decidua of spontaneous and recurrent miscarriages imply a crucial role of the cytokine in the signaling at the feto-maternal interface of the placenta. An overexpression of IL-7 could result in early pregnancy loss by inducing a pro-inflammatory environment. Proven to be valuable in other autoimmune diseases, targeting IL-7 signaling therapeutically may prove to be a very beneficial treatment option for RSA patients.

KEYWORDS
first trimester placenta, interleukin-7, recurrent spontaneous abortion, spontaneous abortion

Theresa Vilsmaier and Niklas Amann equally contributed to this work

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1 | INTRODUCTION

Pregnancy is described as an exceptional immunological state in which the conceptus is a semi-allogenic graft from two different histoincompatible individuals [1] and consequently foreign to the mother. In healthy pregnancy, the maternal immune system adapts and tolerates the semi-allogenic fetus.[2] Immunological balance is fundamental for both maternal and fetal health; nevertheless, immune suppression is essential for a successful pregnancy.[3] In case the maternal immune system is unbalanced, pregnancy may develop pathologically resulting in spontaneous abortion (SA), recurrent spontaneous abortion (RSA), preeclampsia, intrauterine fetal growth restriction (IUGR), and even recurrent embryonic implantation failure.[4–9] SA is defined as one of the most common complication in the first trimester, occurring in 1–2% of human pregnancies before 12 weeks of gestation,[10,11] affecting 25–50% of all reproductive-aged women at least once.[12] RSA is a condition affecting nearly 5% of conceived females (4) and is defined as ≥2 consecutive spontaneous abortions before the 20th week of gestation with the same partner.[13,14] The phenomenon of RSA has been associated with fetal chromosomal abnormalities, endocrine and metabolic dysfunction, anatomical and cytogenetic abnormalities, pro-thrombotic states, autoimmune disorders, and environmental factors as identified causes for RSA.[15,16] For nearly 50% of RSA, the specific etiology remains unexplained.[17,18]

For RSA with unexplained etiology, a maternal immunological rejection of the fetus is discussed as one of the significant factors.[16,19] In early pregnancy, cytokines are key factors in the regulation of immune responses. Various cytokines have been described at the feto-maternal interphase, but there is still limited insight into their pathophysiological input on placentation and embryonic development.[2]

Healthy pregnancy is related to alterations in immune system regulation.[20] Embryonic implantation and labor both act as inflammatory processes and are characterized by the upregulation of T helper 1 (Th-1) and T helper 17 (Th-17) type immune responses.[7,21] A significant amount of women with RSA showed increased levels of peripheral blood natural killer cells (NK) [22,23] and a T helper 1 (Th-1)/T helper 2 (Th-2) immunity imbalance toward increased Th-1 cytokine levels,[5,6] thus resulting in the significantly increased risk for early pregnancy loss.[3,24] Abnormal maternal tolerance to the semi-allogenic fetus is furthermore associated with the T helper 17 (Th-17)/regulatory T-cell (Treg) axis.[25] Th-17 and Treg cells are two different populations; both derive from CD + T cells, nevertheless with reverse effects.[19] Xu et al. described a Th-17/$CD4^+CD25^+$Treg balance shifting toward Treg dominance during normal healthy pregnancy, while an imbalance toward Th-17 dominance is correlated with miscarriage counting RSA.[26] Several studies have described a Th-17/Treg balance shift toward significantly increased Th-17 cells and decreased Treg cells in the peripheral blood and the decidua of women with RSA.[5,8,27] To continue, Treg cell function-related cytokines are reported as advantageous for pregnancy outcome, whereas Th-17 cell function-related cytokines and their pro-inflammatory role at early stage of embryonic implantation are damaging to the development of a healthy pregnancy.[5,25,28,29] An imbalance may induce spontaneous abortion, whereas the balance between these two cell groups is beneficial for a positive pregnancy outcome.[30]

Interleukin-7 (IL-7) is described as a crucial cytokine regulating the proliferation of Th-17 cells.[31] IL-7 is a potent immunostimulatory cytokine of the Interleukin-2 (IL-2) cytokine family and plays a key role in T-cell maturity and homeostasis in humans.[32,33] IL-7 signals via the high affinity IL-7 receptor-alpha chain (IL-7R) by predominantly acting on T cells and inducing potent T-cell proliferation and cytokine production.[34–36] IL-7 is held responsible for the proliferation and persistence of pathogenic Th-17 cells.[5] While Th-17 cells are reported to influence inflammation on embryonic implantation negatively,[28] they are furthermore described to promote various autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, and autoimmune encephalomyelitis by enhancing inflammation.[31,37,38] Hillen et al. were able to describe IL-7 stimulating the production of Th-17 cytokines in a human in vitro model. This activation led to increased arthritis in mice associated with enhanced inflammation and immunopathology. Inhibition of IL-7R in murine experimental arthritis prevented Th-17 cell differentiation, thus leading to decreased severity of arthritis and decreased pro-inflammatory cytokines.[34]

Latest studies have focused on lymphocyte immunotherapy [39] and intravenous immunoglobulin G treatment [40] to modulate the immunological imbalance of Th-17 and Treg cells in women with RSA. Both studies aimed to contribute to a healthy pregnancy by the downregulation of Th-17 cells and upregulation of Treg cells. Although the beneficial effects are still controversial, this approach could be a new therapeutic tool in the future treatment of RSA patients.

Therefore, this project aimed to analyze the expression of IL-7 at the feto-maternal interface of healthy first trimester pregnancies, spontaneous and recurrent spontaneous abortions. Detection of cytokine profiles can contribute to a deeper understanding of the process involved in miscarriage. In the arising importance of immunological therapies, novel understanding could contribute to generating new therapy options.

2 | MATERIAL AND METHODS

2.1 | Patient data

Placental tissue from spontaneous abortions (SA) (n = 18) and recurrent spontaneous abortions (RSA) (n = 17) at gestational weeks ranging from 7 to 14 was obtained and analyzed at the Department of Obstetrics and Gynecology, LMU Munich. RSA was defined as two or more consecutive failed pregnancies (Practice Committee of American Society for Reproductive, 2013). Furthermore, placental tissue from patients undergoing legal termination of a healthy pregnancy (n = 15) in the first trimester served as the control group. The placental material was obtained at a private practice clinic in Munich, Germany.

The placental material was collected after operation without any medical pre-treatment and was acquired by dilatation and uterine curettage. In cases of SA and RSA, the operation was performed within 24 h after diagnosis. Immediately after the curettage, all tissue samples were processed by identical methods and either frozen or formalin fixed for further examination.

Patient's family and medical history were obtained and only those women with an unobtrusive anamnesis were included in our study (Table 1). Exclusion criteria were autoimmune diseases and thrombophilia. Furthermore, patients with microbiological intrauterine infections including bacteria such as Chlamydia trachomatis were excluded. Possible prostaglandin or progestin treatment before operation were identified, and these patients were not included in this study. Chromosomal abnormalities were criteria of exclusion and ruled out by karyotype analysis in all samples, as described before.[41,42] Placental samples from the control group were confirmed as healthy by a blinded independent pathologist.

The study was approved by the Ludwig Maximilian University, Munich Institutional Review Board (Number of approval: 337–06, December 29, 2006) and conducted in accordance with the Declaration of Helsinki. Patients included gave written informed consent before surgery allowing analysis of all clinical and laboratory data.

2.2 | Immunohistochemistry

For immunohistochemistry, the formalin-fixed tissue slides were embedded in paraffin wax. For 15 min, the samples were deparaffinized in xylol and rinsed in 100% ethanol. To inhibit endogenous peroxidase reaction, methanol/H_2O_2 incubation for 20 min was carried out. Later, deescalating alcohol gradients were used on the specimens for rehydration, starting with 100% ethanol and finishing with distilled water. Specimens were cooked in a pressure pot, containing a sodium citrate buffer (pH =6.0) consisting 0.1 mM citric acid and 0.1 mM sodium citrate in distilled water. The samples were then washed twice in PBS, followed by an incubation process for 3 min with a blocking solution (Power Block, diluted 1:10 in Aqua dest, Universal Blocking Reagent, BioGenex). Incubation with the anti- IL-7 primary antibody was performed with each section for 16 h at 4°C (Table 2) at a dilution 1:200. After each following step, samples were washed twice in PBS (pH =7.4). According to the manufacturer's protocol, the reactivity was detected with the Vectastain Elite ABC-Kit (Vector Laboratories). The chromogen-substrate staining was performed for 2 min with the Liquid DAB + Substrate Chromogen System (Dako Scientific). By applying distilled water, the reaction was stopped. The tissue samples were counterstained with Hemalaun for 2 min and blued in tap water at the last step. Specimens were then dehydrated in an ascending alcohol gradient. Lastly, the specimens were cover slipped with Eukitt® quick hardening mounting medium (Sigma Aldrich). Two independent blinded observers evaluated the intensity and distribution pattern of the immunochemical staining reaction by using the semiquantitative immunoreactive score (IRS). The IRS score was defined as follows: a calculation multiplying the percentage of positively stained cells (0, no staining; 1, <10% of the cells; 2, 11–50%; 3, 51–80%; and 4, >80%) with the cell staining intensity (0, none; 1, weak; 2, moderate; and 3, strong). In one circumstance (n = 6,7%), the assessment of the two independent observers varied. Both observers re-evaluated this case together, interpreting the same result at last. The concordance former to the re-evaluation was stated at 91.9%.

Colon tissues were used as positive controls. Negative controls were implemented by replacement of the primary antibodies by

TABLE 1 Demographic and clinical characteristics of the study population

Characteristics	Normal Pregnancy n = 15	Spontaneous Miscarriage n = 18	Recurrent Miscarriage n = 17	p value (Kruskal-Wallis Test)
Maternal age (years)	33.0 ± 6.7 (22.0–41.0)	31.5 ± 8.8 (19.0–43.0)	34.30 ± 4.6 (25.0–39.0)	0.813
Gestational age (weeks)	9.00 ± 2.0 (7–14)	9.84 ± 1.40 (7–14)	8.7 ± 2.2 (7–14)	0.370
Gravidity	3.1 ± 2.0 (1–7)	2.2 ± 2.6 (1–9)	2.9 ± 0.8 (1–4)	0.077
Parity	1.2 ± 1.2 (0–4)	1.2 ± 2.6 (0–8)	0.7 ± 1.08 (0–2)	0.475

Note: Values are given as mean ± SD.; The range is given in parentheses.

TABLE 2 Antibodies used for immunohistochemistry and double-immunofluorescence staining

Antibody	Isotype	Clone	Dilution	Source
IL-7	Mouse IgG1	Monoclonal	1:200, 1:100[a]	R&D Systems, Inc. Minneapolis, USA
Prolactin	Goat IgG	Polyclonal	1:100	R&D Systems, Inc. Minneapolis, USA
Goat-Anti-Mouse Cy3	IgG	Monoclonal	1:500	Dianova, Hamburg, Germany
HLA-G-FITC	Mouse IgG1	Monoclonal	1:50	Serotec, Oxford, UK
Donkey-anti-goat Cy2	IgG	Polyclonal	1:500	Dianova, Hamburg, Germany

[a]Dilution 1:200 for immunohistochemistry, 1:100 for double-immunofluorescence.

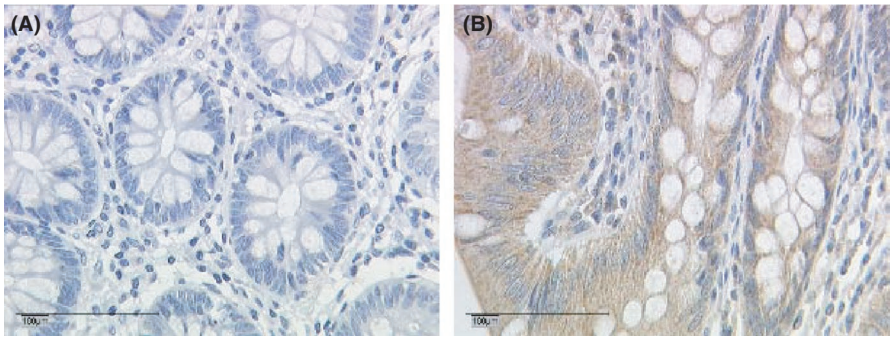

FIGURE 1 Positive and negative control staining. Colon tissue was used for negative (A) and positive (B) control staining of the IL-7 antibody. Scale 100 μm

species-specific isotype control antibodies (Dako) at the same concentration as the primary antibody following the same staining protocol (Figure 1).

2.2.1 | RNA extraction from placental tissue

Total RNA was extracted from placental tissue from specimen included (see Table 1). RNA extraction using 10 mg tissue of each sample was performed with RNeasy® Lipid Tissue Mini Kit (Qiagen, Hilden) according to the manufacturer's protocol. A Nanometer (Implen) was used to quantify the extracted RNA. A ratio of absorbance at 260/280 nm *2.0 was accepted as pure.

2.2.2 | Reverse transcription

High Capacity cDNA Reverse Transcription Kit (Applied Biosystems™, Fisher Scientific Company) was used to perform reverse transcription (RT). This was carried out conferring to the protocol using a master cycler® gradient (Eppendorf,). 300–900 mg/μl templates were used. RT conditions were stated by 10 min at 25°C, 2 h at 37°C, 5 min at 85°C and ending by a hold step at −20°C.

2.2.3 | Gene expression analysis

After RNA was converted to cDNA, quantitative real-time PCR (RT-PCR) amplification was completed with cDNA. Every reaction was carried out with a total volume of 10 μl, including 5 μl cDNA and 5 μl TaqMan® Fast Universal PCR Master Mix 2× (Applied Biosystems, Nr. 4367846; 50 ml) according to manufacturer`s protocol. cDNA at a final concentration of 50 mg per well was applied, according to the recommendation of the manufacturer. Each well enclosed pooled cDNA from both 15 patients with healthy pregnancies and 15 patients with recurrent spontaneous abortions. The temperature protocol was 20 s at 95°C, 40 cycles of amplification, denaturation for 3 s at 95°C, and denaturation plus annealing process for 30 s at 60°C. PCR assay processing was completed with the 7500 Fast Real-Time PCR System (Applied Biosystems). As a housekeeping gene for internal control GAPDH (Hs99999905_m1) was used. Expression levels were examined by the delta-delta CT method ($2^{-\Delta\Delta CT}$).

For identification of differences in cytokine levels in patients suffering from recurrent spontaneous abortion, a gene expression analysis with the Applied Biosystems® TaqMan® Human Immune Response Array (Cat. Nr. 4414073, Applied Biosystems,) was carried out as previously described.[43,44] This panel targets a set of pro- and anti-inflammatory cytokines, including IL-7 (Hs00174202_m1). All assays were plated in triplicates. The gene expression analysis was accomplished with placental tissue from healthy pregnancies and placental tissue from recurrent miscarriage. Patients' placental tissue from spontaneous abortion was not analyzed.

2.2.4 | Double-immunofluorescence staining

To characterize IL-7-expressing cells in the decidua, double-immunofluorescence staining was performed on decidual recurrent abortion tissue. As a specific indicator for extravillous trophoblast cells, HLA-G was used. Prolactin was used to identify decidual stromal cells (Table 2).

Segments were deparaffinized in xylol for 20 min and subsequently washed in ethanol. Further, the slides were incubated in ethanol/methanol for 20 min, rehydrated in an alcohol gradient, and then placed in a pressure cooker with sodium citrate (pH 6.0). The slides were later washed in PBS followed by a blocking process with Ultra V blocking solution (Lab Vision, Thermo Scientific) for 15 min. Following, the sections were incubated with monoclonal anti-IL-7 mouse antibody (dilution 1:100) for 16 hours at 4°C. The slides were then incubated with Cy3 labeled goat-anti-mouse IgG antibody for 30 min at a dilution 1:500 and appearing red. On behalf of the HLA-G staining for indication of extravillous trophoblast, FITC labeled monoclonal anti-HLA-G mouse antibody was applied for 1 h at a dilution 1:50 and later washed in PBS. Polyclonal anti-prolactin goat IgG antibody identified decidual stroma cells, Cy2 labeled donkey-anti-goat antibody, appearing green, was used after incubation for 1 h at room temperature. The slides were then embedded in DAPI containing mounting buffer (Vector Laboratories). Next, the slides were analyzed with a fluorescence Axioskop photomicroscope (Zeiss;) and photographs acquired with a digital Axiocam camera system (Zeiss).

2.2.5 | Statistics

Analysis and statistical data were performed with the SPSS software version 24 (SPSS) and Excel version 12.3.1 (Microsoft Windows 2016). The Mann-Whitney U signed-rank test was performed for the comparison of two independent groups. Kruskal-Wallis test was used for more than two independent groups. *p*-values < .05 were considered to be statistically significant.

3 | RESULTS

3.1 | Real-time PCR with TaqMan® human immune response array

The quantitative real-time PCR identified a 51.4-times higher expression of IL-7 in samples from patients suffering from recurrent miscarriage in comparison with healthy pregnancies (Figure 2A). Gene expression analysis was performed by TaqMan® Human Immune Response Array.

3.2 | Immunohistochemistry

The decidual expression of IL-7 was analyzed in spontaneous abortions (SA, *n* = 18) and recurrent spontaneous abortions (RSA, *n* = 15) in comparison with healthy pregnancies (control group, n = 15). Earlier, positive and negative control staining was performed. Colon tissue was used for negative and positive control staining of the IL-7 antibody. (Figure 1). The decidual stroma cells revealed a significantly higher expression of IL-7 in the RSA group ($p = 0.013$, Figure 3C) compared to the control group with healthy placentas (Figure 3A). Furthermore, a significant upregulation of IL-7 in the decidua of SA placentas ($p = .004$, Figure 3B) was found compared to the control group. RSA had a significantly elevated IRS of 1.28 ± 0.39 and SA of 1.93 ± 0.5 compared to 0.13 ± 0.14 in the control group. The staining results are presented in Figure 3D,2B.

3.3 | Characterization of IL-7-expressing cells at the feto-maternal interphase

Immunofluorescence double staining was able to detect IL-7-positive cells in the decidua of patients with recurrent spontaneous abortion. Decidual stroma cells were identified as IL-7-expressing cells after co-incubation with prolactin (Figure 4). In contrast, the specific marker for extravillous trophoblasts, HLA-G, did not show an expression of IL-7.

4 | DISCUSSION

Maternal immunological rejection of the fetus is discussed as one of the significant factors involved in early pregnancy loss.[16,19] An array of cytokines secreted by both maternal and fetal cells is involved in generating a delicate maternal immune tolerance against the semi-allogeneic fetus during pregnancy,[11] playing a significant role in establishing and maintaining a successful pregnancy. CD4+ T cells are fundamental to this process. CD4+ T cells can be classified into Th-1 cells, Th-2 cells, Treg cells, and Th-17 cells.[7] Previous studies suggested that an imbalance in the Th-1:Th-2 cell equilibrium may result in spontaneous abortion and a healthy pregnancy was believed to be associated with Th-2 dominant immunity.[45] Nevertheless, a Th-2 cell cytokine predominance was later also found in RSA.[46] Consequently, the Th-1:Th-2 paradigm is no longer solely made responsible for the maternal-fetal tolerance, leading to a new hypothesis of Th-1/Th-2/Th-17/Treg cells being involved.[47]

As previously described, detrimental to normal pregnancy is a decrease in Treg cells or an increase in Th-17 cells. In healthy pregnancy, the Th-17:Treg ratio shifts in favor of Treg cells.[48] Nevertheless, the

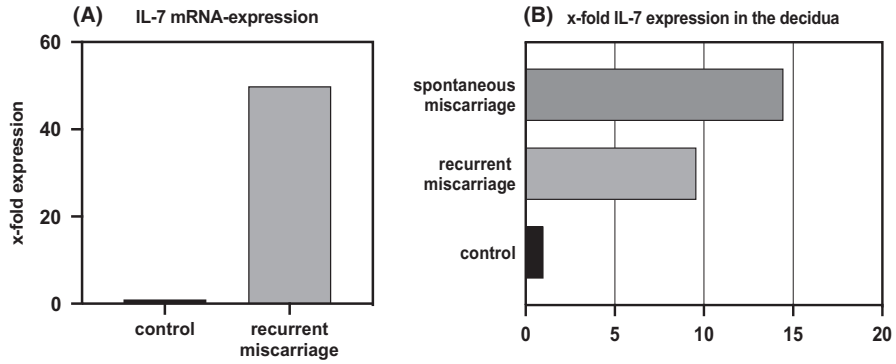

FIGURE 2 (A) Results of quantitative real-time PCR identified a 51.4 times higher expression of IL-7 in samples from patients suffering from recurrent miscarriage compared to healthy pregnancies (individual samples: 46.8; 65.8; 37.0). (B) X-fold expression of IL-7 protein in decidual cells evaluated by immunohistochemistry. IL-7 expression was 9.6 times higher in the decidua of patients with RSA ($p = .013$) and 14.5 times higher in patients with SA ($p = .004$) compared to healthy pregnancies. The bar graphs represent the mean of relative IL-7 expression; the presentation of error bars is not appropriate

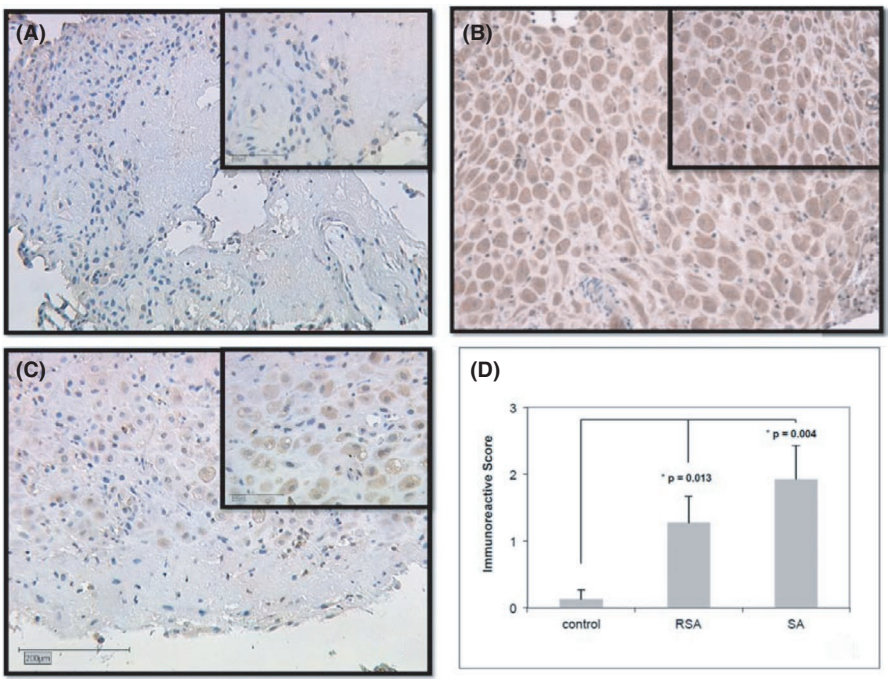

FIGURE 3 (A) In healthy decidual cells, only a weak staining of IL-7 was identified. (B) The decidua of SA specimen showed a significant upregulation (p = .004) of IL-7 in comparison with the control group. (C) Scale 200 μm and 100 μm. The magnified field with the scale of 200 μm in (C) comes from the same specimen as in the 100 μm picture, yet does not appear on the picture. Thus, no arrow on the selected field was included. RSA decidual cells were identified with a significantly higher expression of IL-7 (p = .013). (D) Boxplot analysis of the IRS staining results

function of Th-17 cells in the early stage of pregnancy with RSA is still unclear and has not been fully elucidated. Within this study, we concentrated on the role of IL-7, a key modulator for the differentiation of effector T cells and identified for its particular role inducing Th-17 cell proliferation.[31,49]

Our findings demonstrate a significant upregulation of IL-7 expression in the decidual stroma of patients suffering from spontaneous and recurrent miscarriages compared to normal first trimester pregnancies. IL-7 signals via the high affinity IL-7 receptor-alpha chain (IL-7R) by predominantly acting on T cells and inducing potent T-cell proliferation and cytokine production.[34–36] IL-7 is held responsible for the proliferation and persistence of pathogenic Th-17 cells.[5] As mentioned before, the cytokine IL-7 is involved in T-cell differentiation into Treg or Th-17 cells.[28] IL-7 has been described to induce the differentiation of Th-17 cells by downregulating FOXP3 in Treg cells, thus inhibiting TGF-β-induced Treg differentiation.[11,50,51] Via FOXP3 signaling cascade, Treg cells normally suppress the immune system by downregulating effective T cells.[52] FOXP3, a protein of the nucleus, influences the transcription during DNA-replication. It regulates $CD4^+$-T-effector cells and can inhibit T cell–dependent immune responses.[53] Saifi et al. reported significantly decreased expressions of FOXP3 in unexplained RSA cases compared to healthy pregnancies.[54] Therefore, our findings suggest that during abortion, Treg cells are affected by IL-7. IL-7 might inhibit the expression of FOXP3, thus inducing an imbalanced Th-17:Treg ratio toward a dominant Th-17 immune response. Consequently, resulting in SA or even RSA.

Wang et al. reported significantly increased expressions of Th-17-related cytokines and transcription factor, such as IL-17, IL-23, and RORγt, in the decidua of RSA patients.[19] In contrast, Treg-cell-related cytokines and transcription factor including IL-10, TGF-β, and FOXP3 were significantly decreased.[54,55] Hereafter, the increased Th-17 cells and or deficiency of Treg cells may be responsible for spontaneous abortion, while the balance between these two cells is favorable for healthy pregnancy.[5,30] Hence, IL-7 might play a pivotal role in inducing Treg cell deficiency and inflammatory processes could be stimulated; thus, placental rejection becomes more possible.

Wu et al. investigated a potential mechanism of IL-7 signaling pathway in pregnancy loss by studying IL-7 and IL-7R expressions in decidua of women with RSA.[5] Using an abortion-prone mice model, in vivo IL-7 and IL-7R-antagonist effects on Th-17 and Treg cell populations were explored and correlated with pregnancy outcome. After IL-7R antagonist treatment, Th-17 cells were dramatically decreased and FOXP3 cells were upregulated in mice.[5] This supports our findings of significantly enhanced IL-7 levels in decidua of SA and RSA patients. Consequently, blocking the IL-7/IL-7R signaling pathway could enhance pregnancy outcome by creating a balanced Th-17:Treg cell ratio.

To continue, the secretion from decidualized endometrial stromal cells comprises pro-invasive cytokines including IL-7,[56] which promotes primary trophoblast invasion by activating proteases and altering expression of adhesion-related molecules.[57] IL-7 is reported to increase the expression of human chorionic gonadotropin (hCG), supporting trophoblast's invasion.[58,59] A further study has shown that IL-7R was downregulated in endometrial stromal cells of RSA patients, thus interfering the IL-7/IL-7R signaling pathway resulting in interruption of trophoblast cells successfully invading.[5] A significantly shallower depth of trophoblast invasion has been reported for women with RSA compared to healthy pregnancy.[60] Upregulated IL-7 in SA and RSA decidua cells suggests a compensatory process to decreased IL-7R as similarly reported for other autoimmune diseases [61,62] ; thus, underlining the theory that deregulated IL-7/IL-7R may play a key function in RSA through implantation failure.

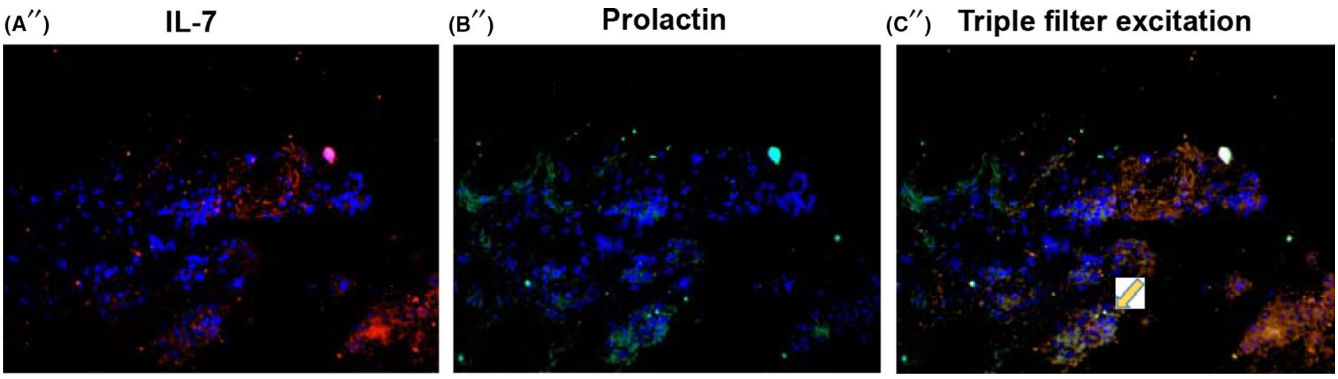

FIGURE 4 (A) IL-7 expression (red) shows a high distribution in decidua of patients with recurrent spontaneous miscarriage. (B) Decidual stroma cells (prolactin positive) appear green. The majority of IL-7-expressing cells are prolactin positive. (C) Extravillous trophoblasts (HLA-G positive) appear green. (D) Triple filter excitation: Arrow indicates double expression of both marker in the decidual stroma. Scale 100 μm

Increased IL-7 is, moreover, reported to promote numerous autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, psoriasis, Sjögrens syndrome, and autoimmune encephalomyelitis, by boosting autoimmune inflammation.[31,37,38] In multiple sclerosis and chronic Chagas disease patients, upregulated IL-7 plasma levels together with reduced IL-7Rα expressions on conventional T cells were reported, without clear genetic influence, concurring to the results of our study.[61,63] Hillen et al. described a human in vitro model in which IL-7 stimulated the production of Th-17 cytokines. This resulted in increased arthritis in mice due to enhanced inflammation and immunopathology. Inhibition of IL-7R in murine experimental arthritis prevented Th17 cell differentiation, thus leading to decreased severity of arthritis, prevented tissue damage, and decreased pro-inflammatory cytokines.[34] Our findings suggest that IL-7 could be one of the key players promoting autoimmune inflammation, consequently playing a significant role in supposedly unexplained RSA patients.

A limitation of this study is that analysis of inter-individual IL-7 mRNA levels cannot be displayed. For the TaqMan® Human Cytokine Network Array, pooled samples from placental tissue of patients with healthy pregnancies and recurrent spontaneous miscarriages were used. As a result, expression of individual IL-7 mRNA levels is unable to be visualized. Nevertheless, the Cytokine Network Array was performed to primarily screen for different cytokine expression levels in miscarriage placentas. IL-7 mRNA expression levels in RSA placentas were 51.4 higher when compared to healthy pregnancies, which was furthermore confirmed by IL-7 upregulation via immunohistochemistry in both miscarriage groups at the protein level. We suggest from a clinical point of view, the inter-individual analysis of protein levels can still be regarded as fundamental since proteins can exert biological function.

To continue, IL-7 was found to be upregulated in both groups of miscarriage; SA and RSA. Consequently, it may not serve as a potential specific biomarker for RSA alone. Differences in the activation pathway of IL-7 in SA and RSA should be observed in the future, especially in regards to the expression of IL-7 receptor and the function of IL-7 and its receptor at the feto-maternal interface.

In conclusion, a significantly increased expression of IL-7 was detected in the human decidua of spontaneous and recurrent miscarriages, suggesting a crucial role in the signaling at the feto-maternal interface of the placenta and immunopathology. An overexpression of IL-7 could lead to a decreased percentage of Treg cells, combined with an increased level of Th-17 cells, provoking immunological imbalance in the Th-17: Treg equilibrium. The induction of a pro-inflammatory environment could result in implantation failure, spontaneous abortion, and unexplained recurrent pregnancy loss. Proven to be valuable in other autoimmune diseases, recently developed anti-IL-7R antibodies, which inhibit signaling of IL-7, may prove to be a very beneficial treatment option for RSA patients, for which further investigations are needed.

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CONFLICTS OF INTEREST

Thomas Kolben holds stock of Roche, relative is employed at Roche, Theresa M. Kolben holds stock of Roche, employed at Roche, Sven Mahner: Research support, advisory board, honoraria and travel expenses from AbbVie, AstraZeneca, Clovis, Eisai, GlaxoSmithKline, Medac, MSD, Novartis, Olympus, PharmaMar, Roche, Sensor Kinesis, Teva, Tesaro. All other authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

T.V., T.K., and U.J. involved in conceptualization. S.L, C.K., and E.S. involved in methodology. S.L. involved in software. T.V., C.K., and U.J. involved in validation. C.K. and S.L. involved in formal analysis. T.V., S.L, and N.A. involved in investigation. S.M. involved in resources. E.S. involved in data curation. T.V. and N.A. involved in writing-original draft preparation. T.K., J.M., A.Z., and J.B. involved in writing-review and editing. T.V., T.K. involved in visualization. U.J. involved in supervision. T.K. involved in project administration. S.M. involved in funding acquisition.

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